Review paper

A new approach to endometrial cancer subtyping – a hope for a milestone in correct patient triaging

Nowe podejście do subtypowania raka endometrium – nadzieja na kamień milowy w prawidłowej segregacji pacjentów

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Abstract

Endometrial cancer is the most common cancer of the female genital organs. For many years the prognosis was based on histopathological grade and type. The pathological identification of endometrial carcinomas included two types. Type I – endometrioid (EEC), similar to the endometrium – was characterized by genetic predisposition, obesity, polycystic ovary syndrome, anovulatory cycles, and irregular menstruation resulting from hyperestrogenism, which is the main predisposing factor for the development of type I EC. Type II included serous, clear cell, and undifferentiated carcinomas. Here were observed older patient's age, higher clinical stage to compare with non-endometrioid histology, and finally poorer prognosis. The Cancer Genome Atlas (TCGA) study found mutations in several endometrioid and serous cancer genes. The TCGA has identified four molecular subclasses based on somatic mutation burden and copy number variations. Recent data show the prognostic value of TCGA subclasses because they correlate with patient survival.

Streszczenie

Rak endometrium jest najczęściej występującym nowotworem żeńskich narządów płciowych. Przez wiele lat podstawą rokowania był stopień dojrzałości histologicznej i typ histopatologiczny. Patologiczna identyfikacja raków endometrium obejmowała jego dwa typy. Typ I – endometrioidalny (EEC), podobny do endometrium, charakteryzuje się predyspozycjami genetycznymi, otyłością, zespołem policystycznych jajników, cyklami bezowulacyjnymi, nieregularnymi miesiączkami wynikającymi z hiperestrogenizmu, który jest głównym czynnikiem predysponującym do rozwoju EC typu I. Do typu II zaliczono: raki surowicze, jasnokomórkowe i niezróżnicowane. Zaobserwowano tu starszy wiek pacjentek, wyższy stopień zaawansowania klinicznego w porównaniu z histologią nieendometrioidalną, a także gorsze rokowanie. Badanie *Cancer Genome Atlas* (TCGA) wykazało mutacje w kilku genach raka endometrioidalnego i surowiczego. TCGA zidentyfikowała cztery podklasy molekularne na podstawie obciążenia mutacjami somatycznymi i zmienności liczby kopii. Najnowsze dane wskazują na wartość prognostyczną TCGA, ponieważ korelują one z przeżyciem pacjentów.

Introduction

Endometrial cancer accounts for close to half of all gynecological malignancies. Pathological examination is a part of diagnostics which may be the basis for the further decision-making process and management. Over the years, the method of treatment changed with the introduction of molecular genetics into diagnostics, in addition to clinical and pathological tests. Numerous authors maintain that the importance of pathology in diagnosis, predicting outcomes and treatment is likely to persist [1, 2]. The FIGO classification created between 1961 and 1971 included the clinical basis for the first time. Since 1988, the classification has included the surgical-pathological data with tumor grade. The histological grading system based only on the proportion of solid and glandular areas is still recommended and used. To improve prognostic evaluation the sentinel node (SLN) mapping method has been included in the National Comprehensive Cancer Network (NCCN) guidelines since 2014. The aim was to improve the identification of lymph node metastases, which are extremely useful in cancer diagnosis, in particular the sentinel nodes or initial nodes of the lymphatic pathways distal to the tumor. This, in turn, could potentially minimize the extent of surgery and associated side effects from extended lymphadenectomy [1, 3].

Histotyping is simple for most endometrial cancers. For high-grade tumors with morphological ambiguity, several systems exist based on surgical-pathological staging. Therefore, incorporating molecular genetics analysis into pathology studies becomes crucial in evaluating the best treatment options. This approach facilitates a more precise and prognostically significant categorization of these cancers [1, 4].

Clinical staging and histotyping in curettage or preoperative biopsy samples may vary with post-surgical specimen examination. An initial curettage diagnosis of endometrioid G1 adenocarcinoma could be corrected to a higher grade of G2 or G3 in the postsurgical specimen. In such cases, there is a risk of underestimation due to non-detection of high-risk tumors. Therefore, additional support by the use of molecular genetics and pathological-genetic classification of endometrial cancer has become important [5].

The TCGA classification

The Cancer Genome Atlas (TCGA) study found mutations in several endometrioid and serous cancers, e.g., TP53, PTEN, PIK3CA, PPP2R1A, FBXW7, CTNNB1, KRAS, and POLE. The asset of this study was to create a simple, cheap and available classification helping with correct triaging [6].

They identified four molecular subclasses based on somatic mutation burden and copy amount changes [7]. The first group includes ultra-mutant endometrial cancer with mutations in the DNA exonuclease epsilon polymerase (POLE) domain. The second group is hypermutated endometrial carcinoma with microsatellite instability. The third group presents high-copy endometrial cancer with frequent TP53 mutation, and finally the fourth group comprises low- and high-copy endometrial tumors. The differences between these groups reached prognostic value and made it possible to explain different outcomes of patients with similar histopathological tumors [8].

Endometrial cancers, termed "ultramutated", are distinguished by pathogenic variants in the POLE exonuclease domain. These mutations in POLE cause misreading during DNA replication, which then leads to a high mutation burden in the endometrium. Approximately 8–10% of all endometrial cancers have one of these POLE mutations. In endometrial cancer molecular classification systems, such cases are referred to as "POLE mutations". They usually occur in relatively young women with early-stage but high-grade tumors with lymphovascular invasion. Despite the high degree of malignancy, POLE mutated tumors are associated with a favorable prognosis and low recurrence rate, regardless of the adjuvant treatment. It is hypothesized that cancer neopeptides caused by an ultramutation may induce a strong cytotoxic immune response. In addition, the ultramutation state may impair the function of POLE mutated tumor cells, leading to a decrease in metastatic potential [9, 10].

The microsatellite unstable group is more commonly referred to as the 'mismatch repair deficient group'. It comprises approximately 25–30% of all endometrial cancers and is defined as the loss of nuclear expression targeted oncoprotein in immunohistochemistry. Moreover, loss of one or more mismatch repair proteins leads to the accumulation of mismatches, insertions, and deletions. Frequently, it is caused by epigenetically driven dysfunction such as hypermethylation of the MLH1 promoter. In a small percentage of cases, it is caused by a germline mutation in one of the mismatch repair genes known as Lynch syndrome. This type of cancer also elicits a strong immunogenic response and has an intermediate prognosis [11].

The third molecular subgroup consists of tumors with a high number of somatic copy number changes and a relatively low percentage of somatic mutations. However, mutations in TP53 are regularly observed, reaching an incidence of up to 90%. This category includes high-grade tumors that generally have a poor prognosis due to their aggressive growth patterns and early propagation. This molecular subgroup is dominated by non-endometrioid histology, most commonly serous adenocarcinoma and about 50% of clear cell carcinomas. Here also are included endometrioid cancers with TP53 mutation, which occur in approximately 61% of cases.

The fourth and the largest subgroup of low copy number endometrial cancers, defined as 'endometrial cancers without a defined molecular profile,' is characterized by a low mutation burden and low somatic copy number variation. The prognosis of these tumors depends on the stage, but it can be considered as intermediate risk. This group usually includes tumors with endometrioid features and expression of estrogen and progesterone receptors. The molecular heterogeneity in this group suggests that further refinement in this group is possible [8, 12, 13].

There may be a worse prognosis in the presence of mutations in exon 3 β-catenin (CTNNB1). They have been identified in 30–50% of endometrial cancers in this subgroup, and the prognosis is relatively poor compared to endometrial cancers without a specific molecular profile without the CTNNB1 mutation [14].

Most endometrial cancers can be precisely classified into one of four molecular subgroups using surrogate markers. 3–6% may fall into more than one classification group, e.g. both abnormal p53 staining and a pathogenic POLE mutation, and are referred to as 'multiple classification endometrial carcinomas'. Recent reports indicate that TP53 mutations may occur as a secondary event to the deficiency of 'mutator' mismatch repair and endometrial carcinoma POLE mutated, without affecting the outcome. Evidence

supports the classification of endometrial cancer with a pathogenic variant of POLE in the exonuclease domain as POLE endometrial carcinoma, regardless of the co-occurrence of a mismatch repair deficiency or abnormal mutant-like p53 immunostaining [12, 13].

Risk factors of endometrial cancer

In addition to the TCGA molecular groups, several other clinicopathological and molecular risk factors have prognostic significance. These include significant (diffuse or multifocal) lymphovascular infiltration, overexpression of L1 cell adhesion molecules, CTNNB1 and 1q32 mutations. L1 cell adhesion molecule is a membrane glycoprotein playing an important role in tumor cell adhesion and migration, strongly associated with TP53 mutation, non-endometrioid histology, high tumor grade, and lymphovascular space involvement. It is an independent risk factor for loco-regional and distant spread. CTNNB1 mutations stimulate the growth of endometrial tissues, which is associated with a higher risk of recurrence and reduced recurrence-free survival [15]. 1q32.1 amplification is associated with a significantly worse prognosis in the subgroup without a specific molecular profile. Our own experience additionally showed the impact of FGFR-2 and also epithelial-mesenchymal transition on outcome.

The ongoing randomized PORTEC-4a study is the first clinical trial to prospectively investigate the use of an integrated clinicopathological and molecular risk profile for the choice of adjuvant therapy. In the study, four molecular subgroups were combined with other prognostic factors (significant lymphovascular space involvement, expression of L1 cell adhesion molecules and CTNNB1 mutation) to determine a favorable, intermediate and unfavorable profile. The PORTEC-4a study is expected to provide important evidence for risk-based treatment selection in patients with high intermediate risk endometrial cancer.

A study of endometrial clear cell carcinomas identified similar genomic classes that were also associated with similar prognosis. Uterine cancer sarcomas also often contain mutations in the TP53, PTEN, PIK3CA, PPP2R1A, FBXW7, and KRAS genes, similar to endometrioid and serous carcinomas [16].

The molecular classification of endometrial cancer is repeatable and has limitations related to clinical outcomes.

The correlation between p53 immunohistochemistry and TP53 copy number changes is not flawless. As a result, the use of this method in these algorithms may lead to misclassification of some high copy number tumors. The algorithms also fail to provide guidance on how to classify tumors with more than one genomic aberration, such as POLE mutations, MMR deficiency, or TP53 mutations, when the components of the algorithm are processed simultaneously rather than sequentially. For example, the ProMisE algorithm performs MMR DNA immunohistochemistry prior to POLE sequencing, which may miss MMR-deficient tumors with POLE mutations and lead to incorrect categorization as MMR-deficient tumors instead of POLE mutations. However, despite these limitations, an integrated approach to genomic-pathological classification, combining genome-based classifications with traditional clinicopathological prognostic factors, remains the most effective method currently available to segregate patients into prognostically different categories that could benefit from personalized treatment options [1, 17, 18].

Undifferentiated endometrial carcinomas, which are rare and highly aggressive neoplasms composed of small to medium-sized cells without noticeable epithelial differentiation, may resemble lymphoma, plasmacytoma, high-grade stromal endometrial sarcoma, or small cell carcinoma. About 40% of these undifferentiated carcinomas are associated with the low-grade endometrioid adenocarcinoma component. At the genomic level, these tumors carry mutations in genes such as POLE, SMARCA4, ARID1B, CTNNB1, PPP2R1A or TP53. A unique subset of endometrioid adenocarcinomas, termed "hyalinized-conducted endometrioid carcinomas" (CHECs), exhibit distinct morphological features such as strings of epithelial cells, spindle-shaped cells, and a stroma that is hyalinized and sometimes forms an osteoid. These tumors are characterized by low malignancy and generally favorable prognosis. Distinguishing them from endometrial carcinomas is essential because the latter tend to occur in older patients and are aggressive malignancies [1, 19].

MSI-H endometrial carcinomas can be identified by assessing morphological features, DNA mismatch repair deficiencies in histology by immunohistochemistry using antibodies directed against MLH1, PMS2, MSH2 and MSH6. There is a strong agreement between the results of immunohistochemistry and PCR-based analysis of microsatellite instability. p53 expression is associated with poor prognosis in endometrial cancer. It correlates with the TP53 mutation status. Identification of POLE mutations in endometrial cancer patients based on tumor morphology and POLE sequencing may help these patients avoid unnecessary treatment given their excellent prognosis. POLE and MSI-H mutation tumors potentially respond well to immunotherapy [1, 20].

For any of the endometrial cancer histotypes, a single marker cannot be a diagnostic tool; therefore it is recommended to use a set of markers containing at least p53 and p16 with ER or PTEN. p16-negative/PTEN-negative and/or ARID1A-negative/p16-negative/p53-wild-type tumors are most likely endometrioid tumors, while serous carcinomas are more likely

to be abnormal p53/p16-positive/ER-negative. In an extended immunohistochemistry panel including DNA mismatch repair proteins (MLH1, PMS2, MSH2, MSH6), the loss of expression of at least one of them supports the diagnosis of endometrioid adenocarcinoma [1, 21, 22].

Excellent results are characteristic of patients with endometrial cancer without high-risk features and with low-grade malignancy. CTNNB1 mutations turned out to be independent predictors of worse recurrence-free survival in groups of patients with endometrial adenocarcinoma. Tumors with CTNNB1 mutations expressed nuclear beta-catenin (a protein product of CTNNB1) [1, 23].

The role of pathology

The role of pathologists in the development and implementation of new therapies is extremely important. In the age of modern oncology, their role includes: identifying homogeneous subsets of cancers that are necessary to obtain meaningful results from molecular/genomic studies aimed at identifying new targets. Evaluation of the expression of molecular biomarkers and their localization at the tissue level can help in making therapeutic decisions. The correlation between phenotype and genotype helps identify tumors with specific molecular targets and amenable to specific therapy. Appropriate patients are selected, based on their phenotypes and biomarker profiles, for participation in clinical trials on new therapies [1, 24].

Patients with the POLE gene mutation have been shown to have a good prognosis and do not require adjuvant treatment. Immunotherapy may apply to a very small percentage of patients with advanced or recurrent disease, and additionally with microsatellite instability and dMMR [25]. Mutated and mismatched repair-deficient tumors exhibit tumor-infiltrating lymphocytes, high levels of neoantigens, expression of immune checkpoint regulators such as programmed death receptor 1 (PD-1) or its ligand PDL-1, which promote escape from immune surveillance. Immune checkpoint blockade with pembrolizumab, an anti-PD1 antibody, has shown a response in patients with endometrial cancer with a POLE mutation and endometrial cancer unable to repair the mismatch. PDL-1 expression can be directly tested in tissues by immunohistochemistry, but optimal methods and antibodies have not yet been standardized [26, 27].

KRAS mutations are common in endometrial cancer and are associated with mucus differentiation. ERBB2 amplifications are also identified in serous endometrial carcinomas. KRAS is not a direct molecular therapeutic target, but the identification of tumors with activation of the MAPK pathway may be amenable to therapy directed against other components of the MAPK/ERK pathway, such as EGFR family members [2, 8].

Conclusions

Numerous *ex vivo*, genomic, translational, pathological, and clinical studies have been conducted over the past years that have greatly advanced the knowledge of endometrial cancer. This has led to refined approaches to diagnosing and treating women with these cancers. As an integral part of any multidisciplinary team, pathology continues to play an important role in diagnosis and prognostic assessment, risk stratification and therapeutic decision making, and the development and implementation of new therapeutic agents and strategies for women with these cancers. According to the report of the World Health Organization, a significant increase in the incidence of endometrial cancer has been observed, emphasizing the need for comprehensive preventive initiatives and careful epidemiological monitoring [1, 28, 29].

Conflict of interest

The authors declare no conflict of interest.

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